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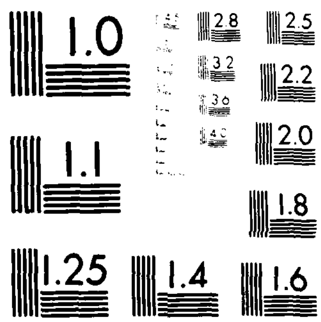

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REPORT ONE

The Control of Larval Dormancy in *Coquillettidia perturbans* (Diptera:Culicidae)

Annual Progress Report

L. P. Lounibos

January, 1980

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-79-C-9093

Florida Medical Entomology Laboratory

P. O. Box 520

Vero Beach, Florida 32961

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Samples of C. perturbans obtained by extracting cattail plants from an abandoned phosphate pit in Mulberry, Florida revealed that winter dormancy occurs in both the third and fourth larval instars. The mean number of larvae extracted per cattail was approximately four times greater in the winter than in the summer, presumed to be due to a firmer attachment of the older larvae in colder weather. Larval densities near to and far from shore did not differ at the site in Mulberry. Pyramidal emergence traps placed over the floating mat of aquatic vegetation provided an effective means of estimating

20. the time of occurrence and density of emerging adult C. perturbans. Both the larval sampling procedure and emergence traps are techniques by which mosquito control workers can monitor populations of this species to determine when and where to apply control measures.

Larvae obtained from collections in the field were cultured under controlled temperature conditions in the laboratory on wheat rootlets. Some pupation was obtained at 26°C, but mortality was unacceptably high. Survivorship improved at 15°C, but larval development was slight. Early indications suggest that 20°C may be the most successful rearing temperature for this species.

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SUMMARY

The current report describes the progress of the initial six months of a study of dormancy in the larval stages of the mosquito Coquillettidia perturbans, a serious pest and potential disease transmitter in North America. The purpose of the research is to provide the biological information that will serve as a foundation for effective control of this mosquito as well as of related species which are important vectors of human filarial worms and viruses in the tropics.

Samples of C. perturbans obtained by extracting cattail plants from an abandoned phosphate pit in Mulberry, Florida revealed that winter dormancy occurs in both the third and fourth larval instars. The mean number of larvae extracted per cattail was approximately four times greater in the winter than in the summer, presumed to be due to a firmer attachment of the older larvae in colder weather. Larval densities near to and far from shore did not differ at the site in Mulberry. Pyramidal emergence traps placed over the floating mat of aquatic vegetation provided an effective means of estimating the time of occurrence and density of emerging adult C. perturbans. Both the larval sampling procedure and emergence traps are techniques by which mosquito control workers can monitor populations of this species to determine when and where to apply control measures.

Larvae obtained from collections in the field were cultured under controlled temperature conditions in the laboratory on wheat rootlets. Some pupation was obtained at 26°C, but mortality was unacceptably high. Survivorship improved at 15°C, but larval development was slight. Early indications suggest that 20°C may be the most successful rearing temperature for this species.

Scientific progress in the initial stages of this project was slowed by administrative delays, changes in the status of the laboratory and its linkages, and the time required to hire personnel and to purchase and install equipment.

FOREWARD

"In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences — National Research Council."

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Body of Report

The mosquito Coquillettidia perturbans experiences a developmental arrest, or diapause, in its larval stages. The current report describes the progress of the initial six months of research supported by DAMD 17-79-C-9093 into the control of larval diapause in this species.

It should be stressed that in spite of its title as Annual, this report covers only the initial six months of funding. Both the slowness inherent in beginning a project on a poorly-studied organism (to the best of my knowledge, there is no other current research project in the U.S. on this important species, due in part to its intractability), and administrative difficulties unique to this period have retarded the execution of this project to the extent originally proposed. In the following paragraph, before proceeding to scientific results, I mention some of the events which have delayed progress in the first six months.

Administrative delays stalled the initiation of the contract until July 1, 1979, and a State of Florida budget, a prerequisite for spending the research funds, was not established until the end of that month. The transfer by the State legislature of the Florida Medical Entomology Laboratory to the University of Florida in July 1979 delayed the authorization of purchase requests. A temporary, summertime employee was hired until the Entomologist II needed for the project was employed in October. Several suppliers were slow in delivering needed equipment. Finally, our linkage in Polk County was temporarily disrupted by the departure of their biologist.

Scientific Results

1.) Diapause Induction in the Laboratory

The execution of these experiments depended on the procurement of eggs of C. perturbans. However, the two principle periods of adult emergence occur in central Florida during the Spring and Summer, before the incubators could be readied for the proposed temperature and light programs (see above), or CDC light traps were purchased for the capture of gravid females. Fulfillment of this phase of the project now awaits the appearance of the Spring 1980 brood of adult C. perturbans. All laboratory and field equipment necessary to perform these experiments are now in place and operational.

2.) Phenology of Natural Populations

The regular sampling of the larval stages of a population of C. perturbans in a single, abandoned phosphate pit in Mulberry, Florida (Polk County) has provided the most substantive data in the early stages of this project. Because the results are best viewed as a contiuum, data that were collected before the inception of the funding period are also included here.

On twenty-five sampling dates between June 1978 and December 1979, fifty or more cattails (Typha latifolia) were removed and processed for C. perturbans larvae according to the methods described in the grant proposal (p. 9). Extracted larvae were staged to instar in the laboratory. Contrary to early expectations, overwintering occurred in both the third and fourth larval instars, and samples in November through February were comprised of 69-87% of

the latter stage (Fig. 1). The hibernal persistence of some third instar larvae at Mulberry differs from the situation reported by Bidlingmayer[1] slightly to the north at Leesburg, Florida where only fourth instar larvae were recovered during the winter. Conceivably, both the milder winters further south and the capacity for more and overlapping generations contribute to the maintenance of two diapause stages at Mulberry.

The first evidences of stage I larvae were collected in April, and in May the percentage of fourth instars decreased as the overwintering generation was presumably depleted by adult emergence. In June and July first and second instars predominated in larval samples, and by late summer and early fall this cohort had largely molted to the third instar. Not until November did the fourth stage become the most common instar in samples. Pupae were recovered only infrequently by the current sampling techniques.

It is probable that two, or even three, broods of C. perturbans occur at Mulberry annually, as determined by analyses of light-trap records (Provost[2]). However, broods are not readily separable from the data on instar distributions, which are complicated by overlapping generations and the relatively slow growth of C. perturbans larvae. Individuals overwintering in the fourth instar would be more likely to contribute to the first emergent brood while larvae diapausing in the third instar may delay emergence until the summer.

In both 1978 and 1979 the density of larvae per cattail was lowest in the summer between July and September, increased during autumn, and reached maxima of four to five larvae per plant between December through February (Fig. 2). This increase in larval densities is associated with the accumulation of fourth instar larvae in the winter (Fig. 1) and a decline in water temperatures. Data from December 1979 through early January 1980 indicate a range in water temperature from 3-15°C at a depth of 40 cm, a level where cattail roots are embedded in the floating mat. (A Weathermeasure recording rainfall gauge and Tempscribe temperature monitor with probe have been mounted on a refurbished surfboard, which has been painted cattail-green for camouflage, and floated on the aquatic mat at the Mulberry site; the rainfall and temperature charts are checked and changed weekly.)

The seasonal changes in recovery of larvae shown in Fig. 2, although repeatable trends, are probably sampling artifacts and not representative of the actual densities of immature C. perturbans at the Mulberry site. Earlier instars, which predominate during the summer (Fig. 1), probably detach more readily from the host plant and may thus be lost during sampling. The attachment of larvae to roots is likely influenced by temperature, and extractions made in the colder months of December and January are less prone to dislodge torpid larvae.

[1]Bidlingmayer, W. L. (1968). Larval development of Mansonia mosquitoes in Central Florida. Mosquito News 28: 51-57.

[2]Provost, M. W. (1976). Mansonia mosquitoes: generations per year in Florida. Fla Anti-mosq. Assoc. Ann. Report 47: 25-27.

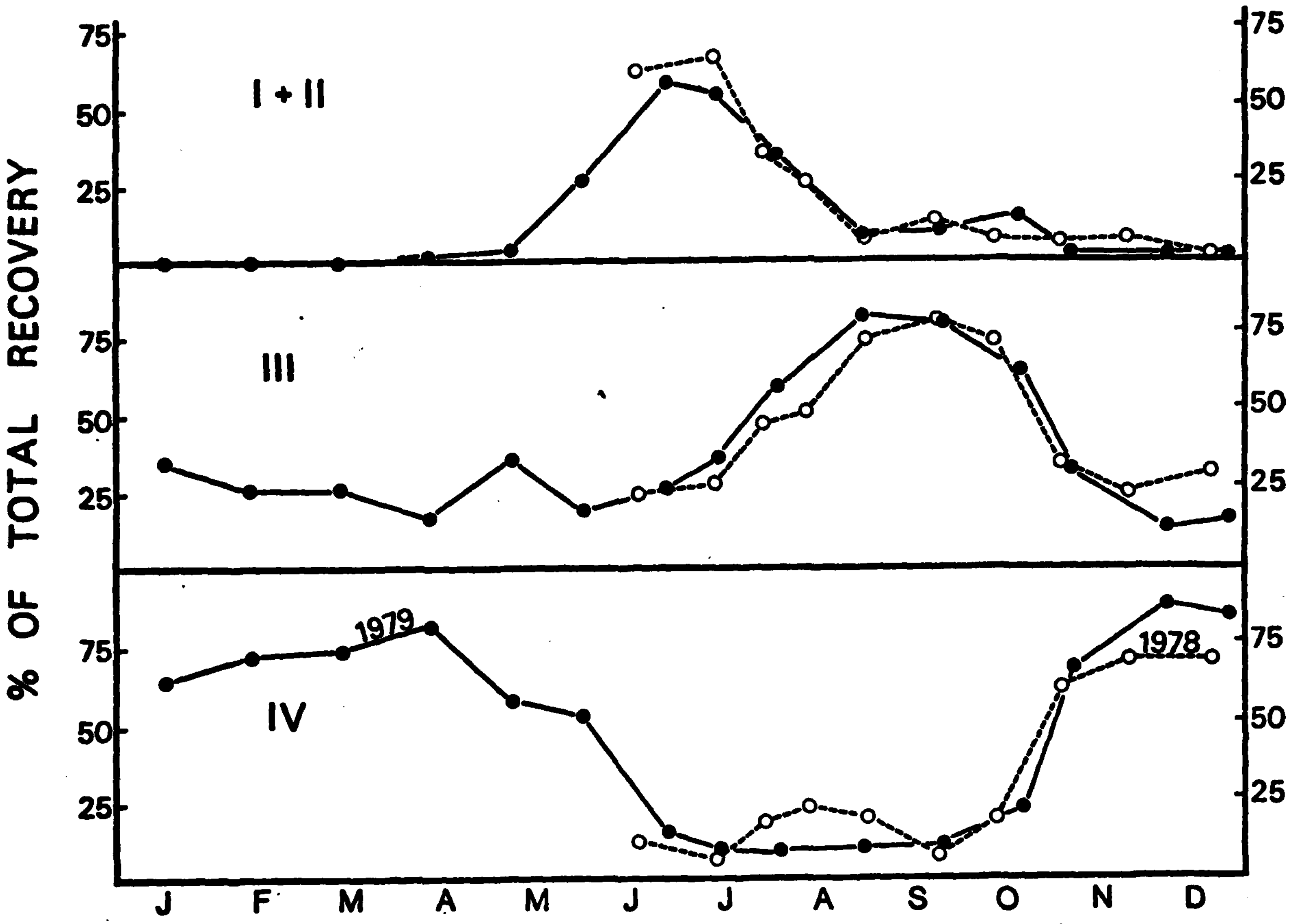


Figure 1. The instar distribution (first and second instars pooled) of larval samples from cattails at the study site in Mulberry, Florida from June, 1978 through December, 1979. each point represents the examination of 100 or more larvae ($\bar{x}$ = 284.5 ± 149.1 SE larvae/sample), excepting the first five sampling dates in 1978 which were composed of between 42-92 individuals.

Two features of the density data from 1979 may seem inconsistent with these explanations. Firstly, larval densities decreased dramatically during March (Fig. 2), although the relative proportions of instars recovered did not change markedly until much later (Fig. 1). The decrease in larval density in March may reflect warming temperatures and a change in the developmental status of the population, possibly pupation. (Although the pupal stage cannot be accurately censused by our extraction procedure, emergence traps indicated the eclosion of adults in early April; see below.) The second inconsistency in the data is the prominent peak in mean number of larvae per cattail observed in June 1979 when second instars predominated. This peak, recorded approximately three to four weeks after a large collection of adults from the emergence trap (see below), may reflect a real increase in the number of C. perturbans larvae in the study site due to ovipositions by the overwintering brood. Continued routine sampling from this site during 1980 will determine if the trends in larval densities observed in 1979 can be expected annually.

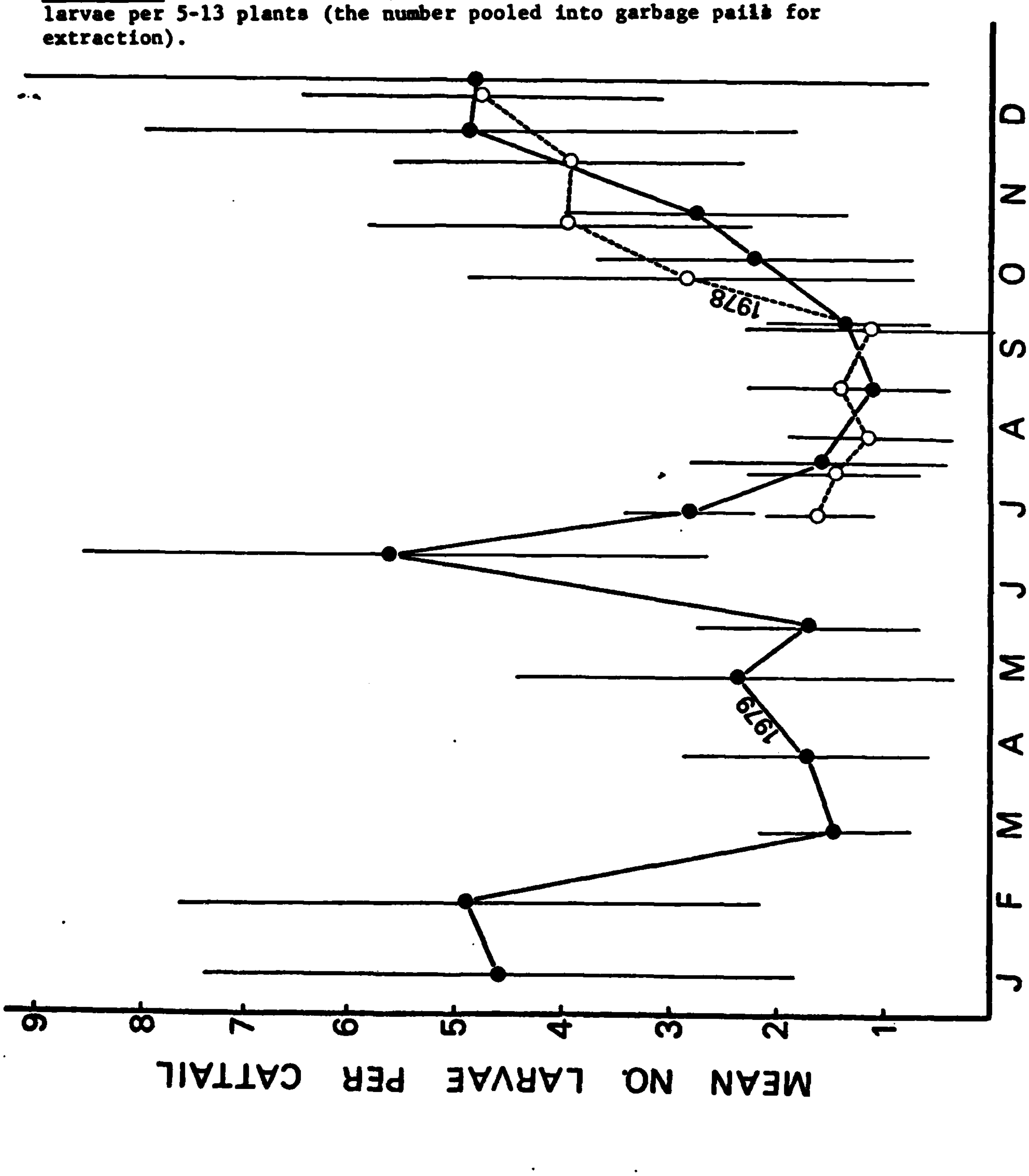
An important consequence of these results is the realization that larval sampling during the winter will provide the best opportunity for assessing if an aquatic habitat is productive of C. perturbans and likely to be a source of adult mosquitoes during the Spring. This information may be of value to mosquito control programs for predicting during the relatively idle winter period which breeding sites may be troublesome later for adult mosquitoes.

For eleven sampling dates between July 1978 and March 1979, cattail plants within 17 meters of the shoreline at Mulberry were separated from plants at least 33 meters distant to determine if larval densities differed near versus far from shore. The mean number of larvae per cattail was 2.91 $\pm$ 1.68 SE near to shore (404 plants sampled) and 2.84 $\pm$ 1.73 SE for the distant collections (489 plants sampled); these means do not differ significantly ($t_s = 0.10$). Thus, at this particular site there is no evidence that larvae are aggregating in one portion of the habitat versus another.

In the Spring of 1979 a prototype emergence trap for the capture of C. perturbans was constructed at Polk County Environmental Services and delivered to the study site at Mulberry. The trap consisted of a screened pyramid on an aluminum frame which measured 2 X 2 meters at its base. An aperture at the apex of the pyramid opened into a removable capture cylinder. On April 10 the device was set at the Mulberry site over aquatic vegetation suspected to harbor C. perturbans, and was checked at irregular intervals for two months thereafter until the upgrowth of plants split the seams of the screen. Adult C. perturbans were recorded at the first check on April 12. Between April 12 and May 3, 48 males and 33 females were recovered alive during a total of six visits to the trap. Between May 7 and June 15, 29 males and 124 females were captured alive on four observation dates; many other specimens were desiccated or had been devoured by ants. These data suggested a peak in emergence, males preceding females, of C. perturbans in May.

These preliminary results led us to conclude that emergence traps can provide an accurate index of the timing of adult eclosion and densities of C. perturbans at the study site. Thus, two additional emergence traps have recently been constructed at Vero Beach and set at the study site in December 1979. No C. perturbans adults have been recorded in the emergence trap during December or early January, nor in a CDC light trap run one night per week at Mulberry since December; both the emergence traps and CDC trap have caught

Figure 2. Seasonal changes in the number of larvae per cattail recovered from the study site in Mulberry, Florida between June 1978 and December 1979. With the exception of four samples in the summer of 1978, each point represents the extraction of eighty or more cattails for C. perturbans. The error term (± 1 SE) was calculated from the number of larvae per 5-13 plants (the number pooled into garbage pails for extraction).



only *Anopheles* and *Culex* during the winter. Rainfall, temperature, and dissolved oxygen recordings were also begun in December, but it is too early for the significance of these initial measurements to be apparent.

3.) Diapause Termination and the Developmental Status of Field Populations

The experiments on diapause termination required the availability of larvae induced to diapause in the laboratory. For reasons explained above, this was not possible in the first six months of this project. Nevertheless, some data were obtained on the developmental status of field populations by observing growth and survival of field-collected larvae on the limited range of temperatures available to the project in its early stages. A summary of these data follows:

Collection Date for Larvae		Developmental Fate
5 Sept., 1979	26°C:	5/13 fourth instar (IV) pupated
		2/41 third instars (III) pupated
		remaining individuals died after 2 1/2 months of culturing
25 Sept., 1979	26°C:	1/15 IV pupated 0/72 III pupated
	8°C:	survivors linger after 3 1/2 months
19 Oct., 1979	26°C:	no IV or III pupated, but one individual collected as a first instar pupated after 1 1/2 months
	15°C:	no development after 3 months of culturing
6 Nov., 1979	26°C:	1/39 IV pupated
		1/45 III pupated
	15°C:	65% survivorship of IV after 2 months, but no signs of development
7 Dec., 1979		survivorship at all temperatures (15°, 20°, 26°) low, the maximum alive one month after culture being 36% at 15°C.
26 Dec., 1979	20°C:	less than 5% mortality after 3 weeks; no development

In sum, there has been considerable mortality and unexplained failures in the laboratory culture of field-collected larvae. The preliminary data suggest that survivorship at 26°C is lower than at 15°C, but at the latter temperature negligible growth is observable. Current experiments are thus relying on incubator temperatures of 20°C. The data from September indicate that summer-collected larvae develop more readily than autumnal collections, irrespective of incubator temperatures, confirming that the field-collected larvae are in a true developmental arrest in the Fall.

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